HIGH SENSITIVITY HUMAN SOLUBLE NEUROPILIN 1 (NRP1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE NRP1 CONCENTRATIONS IN SERUM, PLASMA AND CELL CULTURES

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACK KIT BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HIGH SENSITIVITY NEUROPILIN-1 (NRP1) HUMAN ELISA Kit
Catalog No.	SK00270-06
Lot No.	20115273
Formulation	96 T
Standard range	19.5 ~ 1250 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µL
Dilution Factor	80 ~ 160 (Optimal dilutions should be determined by each laboratory for each application.)
Sample Type	Serum, EDTA plasma, cell cultures
Specificity	Human Soluble NRP1
Calibration	human Soluble NRP1 His Tag (HEK293)
Intra-assay Precision	4 - 8%
Inter-assay Precision	4 - 12%
Storage	2 - 8° C for 8 months. See page 2 for detail
This kit contains sufficient materials to run 35 - 40 samples duplicated provided that assay is run according to protocol.	

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INTRODUCTION

High Sensitivity Human Soluble Neuropilin 1 (NRP1) immunoassay is a solid phase ELISA designed to measure human sNRP1 in serum, EDTA plasma and cell cultures. It contains recombinant the glycosylated human soluble NRP1 derived from HEK293 animal free and antibodies raised against this protein. It has been shown to accurately quantify recombinant human soluble NRP1. Results obtained with naturally occurring soluble NRP1 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human Soluble NRP1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for NRP1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any NRP1 present is bound by the immobilized antibody. After washing away any unbound substances, an antibody biotinylated specific for NRP1 is added to the wells. Following a wash to remove any unbound antibody, Streptavidin-HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of NRP1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

Description	Code	Quantity
NRP1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified Antibody against human NRP1.	**270-06-01**	**1 plate**
NRP1 Standard –40 ng per vial of recombinant glycosylated human sNRP1 His Tag (HEK293) in a buffered protein base with preservative; lyophilized.	**270-06-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL per vial, 10-fold concentrate of purified antibody human NRP1 biotinylated with preservative; lyophilized.	**270-06-03**	**1 vial**
Positive Control Concentrate– one vial of recombinant glycosylated human sNRP1 His Tag (HEK293); lyophilized.	**270-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of SAHRP conjugate with preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB0108CR**	**1 bottle**
HRP Diluent Buffer – 12 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer 20X - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.125M HCl.	**S-STOP**	**1 bottle**

Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 8 months. For longer storage for up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 -8 °C. Do not use kit past expiration date.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (350-400rpm).
- 8-channel Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum or plasma samples may require 80 ~ 160 fold dilution.

A suggested 40 -fold dilution is 10 µL sample + 390 µL 1x Dilution Buffer. The final 80-fold dilution is 50µL per assay well of 40-fold diluted sample plus 50µL per assay well of Dilution Buffer. The final 160-fold dilution is 25 µL per assay well of 40-fold diluted sample plus 75 µL per assay well of Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

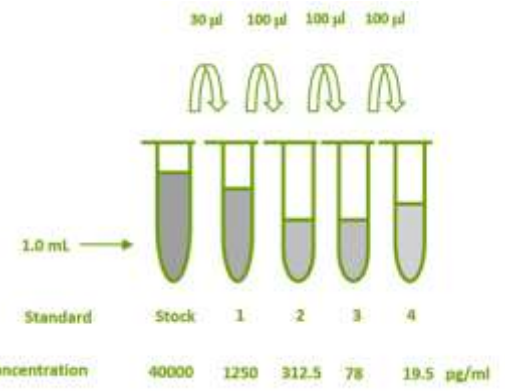
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate 20 X into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

Human NRP1 Standard - Reconstitute the Human NRP1 standard with 1 mL of Dilution Buffer. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1250 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Reconstituted NRP1 standard stock solution can be stored at – 20 ~ - 70 °C for a few days.

Tube	Standard	Dilution Buffer	Concentration
stock	powder	1 mL	40000 pg/mL
# 1	30 µl of stock	930 µl	1250 pg/ml
# 2	100 µl of 1	300 µl	312.5 pg/ml
# 3	100 µl of 2	300 µl	78 pg/ml
# 4	100 µl of 3	300µl	19.5 pg/ml



Positive Control - Reconstitute the **Positive Control** with 1 mL of Dilution Buffer to prepare working solution of Positive Control. Discard the working solution of Positive Control after use.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 ml of **Antibody Diluent Solution (DB0108CR)** to produce a 10-fold concentrated stock solution. For the 96 wells

test, freshly Pipette 9.45 mL of **Antibody Diluent Solution (DB0108CR)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. Reconstituted the Detection Antibody Concentrate (10-fold) can be stored at – 20 ~ - 70 °C for a few days.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Buffer (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution Streptavidin-HRP conjugate (**protect from light**) should be used within 10 min.

Store the 100-fold concentrated stock at 2~ 8 C for 12 months.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Discard each well by hold the plate edge and quickly invert the plate top down. Discard and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a 8-channel Pipettes , manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by discard. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 90 min on microplate shaker at room temperature.
6. Repeat the discard/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP conjugate working solution to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the discard/wash as in step 4
9. Add 100 µL of Substrate Solution to each well. Incubate for 9 ~ 12 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 3 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log or 4-parameter curve fit.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)*
Blank	0 (0.148)
19.5	0.039
78	0.220
312.5	1.029
1250	2.607

Lot No.: 20115273
Positive control: : 100 ~900 pg/mL

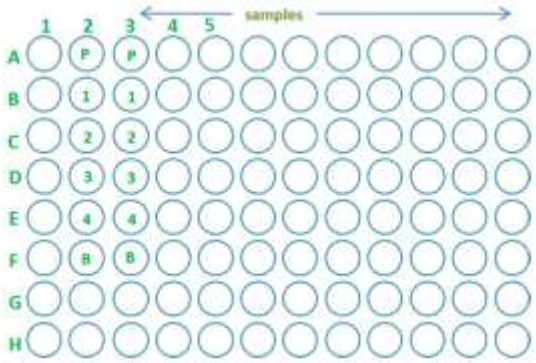
SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Soluble NRP1 (Isoform B) (HEK293)	100
Human NRP2 (HEK293)	0
Human VEGF165	0
Human VEGFR1 (HEK293)	0
Human VEGFR2 (HEK293)	0

Human soluble NRP1 recombinant derived from *E. Coli* or sf21 may not be detected by this ELISA kit.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard dilutions, Pre-diluted samples, or positive control each well. Incubate 2 hours on the plate shaker at RT.
↓
Discard and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate 90 min on the plate shaker at RT.
↓
Discard and wash 4 times.
↓
Add 100 µL Streptavidin-HRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT. **Protect from light.**
↓
Discard and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 9 ~ 12 min on plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 3 min.



Aviscera Bioscience Focus on development new biomarker ELISA Kit for Inflammation, Metabolism, Cardiovascular and Neuroscience Research.

- Human Soluble NRP2 ELISA Kit SK00520-01
- Human Soluble Insulin Receptor ELISA Kit SK00413-06
- Human Raptin/RCN2 ELISA Kit SK00168-11
- Mouse RCN2 Raptin ELISA Kit SK00168-12
- Human Soluble VEGF-R1/FLT-1 ELISA Kit SK00114-01
- Human PEDF ELISA Kit SK00283-06
- Human BD-1 ELISA Kit SK00858-06
- Human BD-3 ELISA Kit SK00857-06
- Human BD-2 ELISA Kit SK00044-01
- Human BD4 ELISA Kit SK00853-01
- Human BD5 ELISA Kit SK00854-01
- Human Plasma Gesolin ELISA Kit SK00384-01
- Human VDBP ELISA Kit SK00627-01
- Human Soluble CD209 ELISA Kit SK00345-09
- Lipopolysaccharide Binding Protein (LBP) (Human) ELISA Kit SK00248-01
- Human Azurocidin/CAP37/HBP ELISA Kit SK00832-02
- Human CSF1R ELISA Kit SK00144-06
- Human TREM2 ELISA Kit SK00218-12A
- Human Cholesin/C7ORF50 ELISA Kit SK00027-09
- Human Endotrophin/COL6A3 C Terminal Peptide ELISA Kit SK00009-06
- Human Soluble ACE2 ELISA Kit SK00707-06